Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein, is respectfully requested. Claims 1 and 28 are amended, claims 3-8 are cancelled, and claims 29-30 are added; as a result, claims 1-2 and 9-30 are pending in this application. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment, which claims are present in a continuation of the present application.

The Examiner is respectfully thanked for the courtesies extended to Applicant's Representatives in the telephonic interview on March 10, 2004.

The amendments to claim 1 and 28 are supported by the specification at page 7, line 34 through page 9, line 13, page 26, lines 9-18, page 33, lines 19-22, and page 39, lines 18-21.

New claims 29 and 30 are supported at page 33, lines 23-25 and page 39, lines 22-24 of the specification.

A certified translation of the priority document for the present application is enclosed herewith.

The 35 U.S.C. § 102(b) Rejection

The Examiner rejected claims 1-2, 6, 9-13, 15-17, 19-23, and 28 under 35 U.S.C. § 102(b) as being anticipated by Guillot et al. (WO 99/18234). This rejection is respectfully traversed.

Applicant respectfully submits that WO 99/18234 is not available as a reference under 35 U.S.C. § 102(b), but is available as a reference under 35 U.S.C. § 102(a). For the sake of expediting prosecution of the instant application, if the Examiner were to present the rejection under 35 U.S.C. § 102(a), Applicant provides the following remarks. Additionally, Applicant maintains the right to swear behind any reference which is cited in a rejection under 35 U.S.C. §§102(a), 102(e), and/or 103.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Dillon*, 919 F.2d 688, 16 U.S.P.Q.2d 1897, 1908 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991). For anticipation, there must be no difference

between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the art. *Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 18 U.S.P.Q.2d 101 (Fed. Cir. 1991).

Guillot et al. generally disclose a method in which microorganisms potentially present in a sample, e.g., a sample which has been fixed with an agent that maintains morphological integrity, are contacted with a RNA-targeted oligonucleotide probe, and the hybridized probes extracted, detected, and measured (page 4, lines 6-16 and page 6, lines 10-14). The extraction step is "performed by placing the microorganisms potentially present under conditions to denature enabling the denaturation of every probe specifically associated with its target sequence, notably in the presence of a probe-target denaturing agent such as one that will separate duplex DNA/DNA or DNA/RNA, and in particular the probe-target duplex under consideration, and at a temperature higher than the melting temperature of the probe under consideration, notably at a temperature of about 100°C" (page 8, lines 5-11). Guillot et al. continues by disclosing that the preferred denaturing agent is formamide and that extraction with formamide is performed at 100°C (page 8, lines 11-13). No other denaturing agents or protocols for detecting microorganisms in a sample are disclosed in Guillot et al. On the contrary, the advantages of formamide in Guillot et al. are stressed at, for example, page 8, lines 11-13. Therefore, Guillot et al. provide an explicit teaching to use formamide as a denaturing agent, and do not provide alternatives for formamide or disclose disadvantages associated with the use of formamide.

Applicant respectfully asserts that while Guillot et al. generally disclose the use of denaturing agents to extract hybridized probes, there is nothing in the Guillet et al. reference that teaches the separation of hybridized nucleic acid probe molecules from target nucleic acid in water, DMSO, 1 X SSC or 0.001-0.01 M Tris/HCl, pH 9.0 +/- 2.0 at a temperature that provides a stronger signal from separated nucleic acid probe molecules than from corresponding nucleic acid molecules separated using formamide.

Accordingly, the Examiner is respectfully requested to withdraw the rejection under 35 U.S.C. § 102.

The 35 U.S.C. § 103(a) Rejections

The Examiner rejected claims 1-13, 15-17, 19-23, and 28 under 35 U.S.C. § 103(a) as being unpatentable over Guillot et al. in view of the Appendix of Roe et al. (Recombinant DNA Isolation, Cloning and Sequencing, 1996) and further in view of Kemp et al. (U.S. Patent No. 6,090,627). The Examiner also rejected claims 1-2, 6, 9-23, and 28, under 35 U.S.C. § 103(a) as being unpatentable over Guillot et al. in view Sanders et al. (U.S. Patent No. 5,888,725). The Examiner further rejected claims 1-24 and 28 under 35 U.S.C. § 103(a) as being unpatentable over Guillot et al., in view of Roe et al., Kemp et al., and Sanders et al. These rejections, as they may be maintained with respect to the pending claims, are respectfully traversed.

The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). To establish a *prima facie* case of obviousness, three criteria must be met. First, the prior art reference (or references) must teach or suggest all of the claim limitations. Second, there must be some suggestion or motivation, either in the cited reference (or references), or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Third, there must be a reasonable expectation of success. M.P.E.P. § 2142 (citing *In re Vaeck*, 947 F.2d 488. 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

Guillot et al. do not disclose or suggest the separation of hybridized nucleic acid probes from their target in water, DMSO, 1 X SSC or 0.001-0.01 M Tris/HCl, pH 9.0 +/- 2.0 at a temperature that provides a stronger signal from detectable separated nucleic acid probe molecules than from corresponding hybridized nucleic acid molecules separated using formamide. Moreover, Guillot et al. teach that formamide is the preferred denaturing agent (see page 8, lines 11-13). Thus, Guillot et al. teach away from the present invention.

The portion of Roe et al. relied on by the Examiner in the Appendix of Roe et al. discloses a 10X denaturing buffer containing 200 mM Tris-HCl, pH 9.5, 1 mM EDTA, and 10 mM spermidine in double distilled water. From the title of Roe et al., "Protocols for Recombinant DNA Isolation, Cloning, and Sequencing," it appears that the solutions listed therein are for use in recombinant DNA isolation, cloning, and sequencing.

The Appendix of Roe et al. does not disclose or suggest whether the solutions disclosed therein are suitable for use in other protocols, e.g., in a method of detecting nucleic acids of fixed

microorganisms and, in particular does not teach or suggest the use of 200 mM Tris-HCl, pH 9.5, 1 mM EDTA, and 10 mM spermidine in double distilled water to separate hybridized probe molecules from a target.

Kemp et al. disclose the sequence of the T-DNA of the octopine-type Ti plasmid found in *Agrobacterium tumefaciens* ATCC 15955, and the use of promoters and polyadenylation sites from pTi15955 to control expression of foreign structural genes (abstract). To sequence the T-DNA of pTi15955, Kemp et al. disclose that fragments of the T-DNA were subcloned into pBR322, and individual clones sequenced after the resulting plasmid DNA was digested, treated with calf intestinal phosphatase, denatured in 20 μM Tris-HCl, pH 9.5, 1 mM spermidine and 0.1 mM EDTA, and phosphorylated with kinase in the presence of radioactive ATP (column 28, lines 8-28).

Kemp et al. do not teach or suggest a method of detecting the nucleic acid of fixed microorganisms. Nor do Kemp et al. disclose or suggest that 20 μM Tris-HCl, pH 9.5, 1 mM spermidine and 0.1 mM EDTA may be employed as a separation solution to separate hybridized probe molecules from a target.

Sanders et al. disclose "[a] method for detection, identification and/or quantification of target organisms of specific bacterial genus, species or serotypes, based on the occurrence of release of cell contents, particularly nucleotides, e.g., ATP, on lysis of bacterial cell walls on incubation with bacteriophages (phages) specific for them" (abstract). No cellular contents are disclosed other than NAD, NADP, NADH, NADPH, ATP, ADP, cAMP, or cGMP (column 2, lines 32-45).

Sanders et al. do not disclose or suggest a method of detecting microorganisms in a sample by hybridization of microbial nucleic acids with a nucleic acid probe.

Clearly, Roe et al., Sanders et al., and Kemp et al. do not remedy the deficiencies of Guillot et al. as none of the cited art teaches or suggests a method in which hybridized nucleic acid probes are separated from their target in water, DMSO, 1 X SSC or 0.001-0.01 M Tris/HC1, pH 9.0 +/- 2.0 under conditions that provide a stronger signal from detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated from their target using formamide.

The Examiner asserts that it would have been obvious to one skilled in the art at the time the invention was made to have practiced the claimed method taught by Guillot et al. of using a denaturing agent in step d), wherein the denaturing agent was contained in the denaturing buffer of Roe et al., in order to provide a solution with double distilled water as the denaturing agent in conjunction with the use of Tris/HCl concentrations and the proper pH as exemplified by Kemp et al. with which to denature the hybridized probes for subsequent, accurate quantification "as the Tris/HCl solution is equally as effective means to release the bound probes" (emphasis added, page 7 of the Office Action). The Examiner also asserts that it would have been obvious to augment the Tris/HCl concentration and temperature of the separation solution within the limits taught by Roe and Kemp et al. as optimization of conditions for performing a method step are well within the skill of the art.

Nevertheless, the Examiner has not identified any suggestion or motivation in the cited art to combine Guillot et al., Roe et al., Sanders et al., and Kemp et al. in a manner necessary to arrive at the instant claims. Applicant respectfully submits that there is no motivation to combine Guillot et al. with any of Roe et al., Sanders et al., or Kemp et al. because Roe et al., Sanders et al., and Kemp et al. are from non-analogous art. Guillot et al. relate to detection of microorganisms in a sample with a nucleic acid probe, while Sanders et al. relate to detection of microorganisms via release of cellular contents, Roe et al. relate to recombinant DNA isolation, cloning, and sequencing, and Kemp et al. relate to sequencing and genetic engineering of pTi15955 DNA.

Moreover, the Examiner is using knowledge of Applicant's disclosure ("as the Tris/HCl solution is equally as effective means to release the bound probes") to support the rejection as it is only Applicant's specification that teaches that certain separation solutions are better than those with formamide.

Further, even if, for the sake of argument, one skilled in the art did combine the references as suggested by the Examiner, there would be no definitive teaching on what separation solution to employ and, in particular, what separation solutions and conditions would result in more signal from separated nucleic acid probes relative to the signal from corresponding hybridized nucleic acid molecules separated using formamide.

Finally, it is submitted that the cited documents would not have provided one skilled in the art with a reasonable expectation that the instantly claimed methods for detecting a microorganism in a sample could yield greater signal. Such a result was unexpected.

The Examiner is also requested to consider the Rule 132 Declaration enclosed herewith. In the Declaration Dr. Snaidr summarizes results from experiments in which separation was conducted at 80°C with 0.01 M Tris-HCl, pH 9.0, water, 1 x SSC, pH 10.0, DMSO, or formamide, or conducted at 100°C with 0.01 M Tris-HCl, pH 9.0, water or formamide. For all of the tested conditions, the non-formamide-based separation solutions yielded a stronger signal than formamide separation solutions. Dr. Snaidr also provides results for the signal obtained over time when Cy3-labeled oligonucleotides were diluted in water, 0.01 M Tris-HCl, pH 9, DMSO or formamide (Figures 1-2 in the Declaration). He points out that the fluorescent signal from formamide diluted oligonucleotides was 80% lower than the signal from oligonucleotides diluted in water (paragraph 4 of the Declaration). Moreover, as shown in Figure 1, at 80°C, the signal intensity from formamide diluted oligonucleotides declines rapidly in contrast to the signal intensity obtained from non-formamide based solutions (Figures 1-2). Dr. Snaidr concludes that the use of non-formamide based separation solutions within the scope of the claims to denature target hybridized probes with detectable signals yields a stronger signal than the use of a formamide separation solution.

Therefore, Applicant respectfully requests the Examiner to withdraw the rejections under 35 U.S.C. § 103(a).

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney ((612) 373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743

Respectfully submitted,

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Date May 24, 2004

By _____
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Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 24th day of May, 2004.

Patricia A. Hultman _____

Name Signature